PTO 09-7332

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20010103
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A THROMBOLYTIC MEDICATION, ITS PREPARATION METHOD AND ITS USE
[Yi zhong rong shuan yao wu, qi zhi bei fang fa ji qi yong tu]

Jikai Liu et al.

UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. AUGUST 2009
TRANSLATED BY: THE MCELROY TRANSLATION COMPANY

PUBLICATION COUNTRY	(19):	CN
DOCUMENT NUMBER	(11):	1278433
DOCUMENT KIND	(12):	A
PUBLICATION DATE	(43):	20010103
APPLICATION NUMBER	(21):	00112893.0
APPLICATION DATE	(22):	20000426
INTERNATIONAL CLASSIFICATION[7]	(51):	A 61 K 35/78 A 61 P 7/02 A 61 P 9/10
INVENTORS	(72):	Jikai Liu et al.
APPLICANT	(71):	Kunming Plant Research Institute, Chinese Academy of Sciences Co-Applicant Kunming College of Medicine
TITLE	(54):	A THROMBOLYTIC MEDICATION, ITS PREPARATION METHOD AND ITS USE
FOREIGN TITLE	[54A]:	Yi zhong rong shuan yao wu, qi zhi bei fang fa ji qi yong tu

Claims /1*

1. A thrombolytic medication, characterized in that it uses *Phyllanthus urinaria* (chamberbitter) or *Phyllanthus cochinchinensis* (Vietnam leafflower) or *Phyllanthus emblica* (Indian gooseberry) or *Phyllanthus simplex* (virgate leafflower) or *Phyllanthus matsumurae* (Matsumurae leafflower) as the raw material to prepare a medication using the following methods:

Phyllanthus urinaria or *Phyllanthus cochinchinensis* or *Phyllanthus emblica* or *Phyllanthus simplex* or *Phyllanthus matsumurae* is dried and pulverized to obtain a dry powder, extracted 2-4 times using ethanol, industrial alcohol, hydrous acetone or any of their solvents at room temperature, the extraction solution is vacuum condensed at 30-40°C until dry to obtain an extract, the extract is suspended using n-butyl alcohol, ethyl acetate or n-butyl alcohol containing a small quantity of organic acid, it is extracted fractionally using water, the water layers merged, and vacuum condensed at 40°C to a small volume, then spray drying is performed to obtain a dry powder; it to form a solid preparation dose, the dry powder is combined with pharmaceutically acceptable carriers and/or excipients at a weight ratio of 0.1:99.9 to 99:1; or it is combined to form a liquid preparation dose using routine preparation methods.

2. A thrombolytic medication preparation method according to Claim 1, characterized in that the *Phyllanthus urinaria* or *Phyllanthus cochinchinensis* or *Phyllanthus emblica* or *Phyllanthus simplex* or *Phyllanthus matsumurae* is dried and pulverized to obtain a dry powder, extracted 2-4 times using ethanol, industrial alcohol, hydrous acetone or any of their solvents at room temperature, the extraction solution is vacuum condensed at 30-40°C until dry to obtain an extract, the extract is suspended using n-butyl alcohol, ethyl acetate or n-butyl alcohol containing a small quantity of organic acid, extracted fractionally using water, the water layers merged, vacuum condensed to a small volume at 40°C, then spray drying performed to obtain a dry powder, to form a solid preparation dose, it is combined with pharmaceutically acceptable carriers and/or excipients at a weight ratio of 0.1:99.9 to 99:1.

* [Numbers in right margin indicate pagination of the original text.]

3. A thrombolytic medication preparation method according to Claim 1, characterized in that the *Phyllanthus urinaria* or *Phyllanthus cochinchinensis* or *Phyllanthus emblica* or *Phyllanthus simplex* or *Phyllanthus matsumurae* is dried and pulverized to obtain a dry powder, extracted 2-4 times using ethanol, industrial alcohol, hydrous acetone or any of their solvents at room temperature, vacuum condensed at 30-40°C until dry extract is obtained, the dry extract suspended using n-butyl alcohol, ethyl acetate or n-butyl alcohol containing a small quantity of organic acid, extracted fractionally using water, the water layers merged, vacuum condensed at 40°C to a small volume, then spray drying is performed to obtain a dry powder, it is combined to form a liquid preparation dose according to routine preparation methods for liquid preparations.

4. A medication according to Claim 1, used to prepare a medication to treat thromboembolisms.

5. A medication according to Claim 1, used to prepare a medication to treat myocardial infarction.

6. A medication according to Claim 1, used to prepare a medication to treat hypertension.

7. A medication according to Claim 1, used to prepare a medication to treat angina pectoris.

8. A medication according to Claim 1, used to prepare a medication to treat stroke.

9. A medication according to Claim 1, used to prepare a medication to treat cerebral thrombosis.

10. A medication according to Claim 1, used to prepare a medication to treat pulmonary embolism.

11. A medication according to Claim 1, used to prepare a medication to treat limb arteriovenous embolisms.

12. A medication according to Claim 1, used to prepare a medication to treat retinal arteriovenous embolisms.

The present invention pertains to the field of pharmaceutical technology, specifically speaking, it /1
involves the use of *Phyllanthus urinaria* or, from the same plant genus, *Phyllanthus cochinchinensis* or *Phyllanthus emblica* or *Phyllanthus simplex* or *Phyllanthus matsumurae* as the raw material to prepare a thrombolytic medication, preparation method for said medication, and its use in the pharmaceutical field.

Statistical data demonstrates that thrombus- and atherosclerosis-induced cardiovascular system disease is one of the main causes of death and disease in the human population. Thrombolytic disease (cerebral, cardiac, and limb embolisms, among others) incidence rates are high, and cerebral stroke and myocardial infarction are the chief causes of death in the population. Some 70% of cerebral strokes are cerebral thrombosis. Almost 100,000,000 patients require high-efficacy, low-cost anticoagulant, thrombolytic, blood-activating medications for treatment. The balance between tPA (tissue-type plasminogen activator) and PAI-1 (plasminogen activator inhibitor-1) is critical to thrombus formation and elimination. The interaction between these two, the regulation of high and low PAI-1 activity in plasma, constitutes the entire process of fibrinolysis (Dawson et al., Atherosclerosis 1992, 95:105). Clinical and experimental data indicate that elevated PAI-1 activity in plasma is an extremely high risk factor, it may reduce the blood's fibrin degradation functionality, thereby inducing a host of cardiovascular diseases, such as myocardial infarction and coronary artery thrombopoiesis (Charlton, Drug of the Future 1997, 22:45). Thus, inhibiting PAI-1 activity is an extremely important treatment target. Finding a small-molecule PAI-1 inhibitor will enable the discovery of a novel high-efficacy thrombolytic medication. At present, thrombolytic medications in clinical use have a common defect, hemorrhagic complications. Additionally, cost of treatment is high, medication half-life is short, and because said medications are large molecules, they very often induce allergies. Because of its unique mechanism of action, a highly selective PAI-1 inhibitor will make it possible to avoid this side effect. In recent years, major pharmaceutical manufacturing groups in developed nations in the United States and

Europe have been extremely interested in this field of research, yet to date only a small number of small-molecule PAI-1 inhibitors have been discovered. The Xenova Company in England has a microbe-sourced compound; the company has already completed animal testing *in vivo* and *in vitro* and it has already gone into clinics (Xenova). On this basis, the development of new, natural small-molecule PAI-1 inhibitors and their use as novel thrombolytic medications to treat, prevent and achieve recovery from thromboembolic disease is a new thrombolytic medication pathway for the treatment of myocardial infarction, hypertension, angina pectoris, stroke, cerebral thrombosis, pulmonary embolism, limb arteriovenous embolism, retinal arteriovenous embolism and other diseases. To date, existing technology using *Phyllanthus urinaria* or, from the same plant genus, *Phyllanthus cochinchinensis* or *Phyllanthus emblica* or *Phyllanthus simplex* or *Phyllanthus matsumurae* as the raw material for an ethanol-extraction product as the effective part or active ingredient to prepare a thrombolytic medication has not yet been reported.

The objective of the present invention is to supply an ethanol extraction product of *Phyllanthus* /2 *urinaria* or from the same plant genus, *Phyllanthus cochinchinensis* or *Phyllanthus emblica* or *Phyllanthus simplex* or *Phyllanthus matsumurae* to serve as the effective part for preparing a thrombolytic medication, and also to supply said medication's preparation method, and its use in preparing thromboembolic medication, myocardial infarction medication, hypertension medication, medication to treat angina pectoris, medication to treat stroke, cerebral thrombus medication, pulmonary embolism medication, limb arteriovenous embolism medication and retinal arteriovenous embolism medication.

The present invention supplies the following technical protocols to achieve the objectives in the present medication:

A thrombolytic medication using *Phyllanthus urinaria* or *Phyllanthus cochinchinensis* or *Phyllanthus emblica* or *Phyllanthus simplex* or *Phyllanthus matsumurae* as the raw material to prepare a medication using the methods below:

Phyllanthus urinaria or *Phyllanthus cochinchinensis* or *Phyllanthus emblica* or *Phyllanthus simplex* or *Phyllanthus matsumurae* is dried and pulverized to obtain a dry powder, is extracted 2-4 times at room temperature using ethanol, industrial alcohol, hydrous acetone or any of its solvents, vacuum condensed at 30-40°C until dry to obtain the extract, the extract is suspended using n-butyl alcohol, ethyl acetate or n-butyl alcohol containing a small quantity of organic acid, it is extracted fractionally using water, the water layers merged, it is vacuum condensed at 40°C to a small volume, then spray drying performed to obtain a dry powder, the dry powder is combined with pharmaceutically acceptable carriers and/or excipients at a weight ratio of 0.1:99.9 to 99:1 to form a solid preparation dose; or using routine preparation methods, it is combined to form a liquid preparation dose.

The present invention further supplies a preparation method for the thrombolytic medication described above, using *Phyllanthus urinaria* or *Phyllanthus cochinchinensis* or *Phyllanthus emblica* or *Phyllanthus simplex* or *Phyllanthus matsumurae*, which is dried and pulverized to obtain a dry powder, extracted 2-4 times using ethanol, industrial alcohol, hydrous acetone or any of its solvents at room temperature, vacuum condensed at 30-40°C until dry to obtain the extract, the extract suspended using n-butyl alcohol, ethyl acetate or n-butyl alcohol containing a small quantity of organic acid, extracted fractionally using water, the water layers merged, vacuum condensed at 40°C to a small volume, then spray drying performed to obtain a dry powder, the dry powder is combined with pharmaceutically acceptable carriers and/or excipients at a weight ratio of 0.1:99.9 to 99:1 to form a solid preparation dose.

The present invention supplies an additional preparation method for the thrombolytic medication described above, using *Phyllanthus urinaria* or *Phyllanthus cochinchinensis* or *Phyllanthus emblica* or

Phyllanthus simplex or *Phyllanthus matsumurae*, dried and pulverized to obtain a dry powder, extracted 2-4 times using ethanol, industrial alcohol, hydrous acetone or any of its solvents at room temperature, vacuum condensed at 30-40°C until dry to obtain an extract, the extract is suspended using n-butyl alcohol, ethyl acetate or n-butyl alcohol containing a small quantity of organic acid, extracted fractionally using water, the water layers merged, vacuum condensed at 40°C to a small volume, spray drying performed to obtain a dry powder, then it is combined to prepare a liquid preparation dose using routine preparation methods.

The present invention further supplies applications for the thrombolytic medication described above for preparation of thromboembolic medication, myocardial infarction medication, hypertension medication, angina pectoris medication, stroke medication, cerebral thrombus medication, pulmonary embolism medication, limb arteriovenous embolism medication and retinal arteriovenous embolism /3 medication.

The effective part of the above-described plants for a medication can be used alone or in combination. Said medication compound contains 0.1-99%, preferably 0.5-90%, effective part, and the rest of the compound is pharmacologically acceptable: pharmaceutically acceptable carriers and/or excipients that are not toxic to people or animals.

One or more of the described carriers are selected from solid, semi-solid and liquid diluents, fillers and supplemental medication preparation agents, the described medication compound is used in administration dosage by unit weight. The medication in the present invention can be administered in two ways, orally or injected (intravenously and intramuscularly).

The oral medication may be taken in its solid or liquid preparation, such as powder, tablet, sugar-coated, capsule, granular floating solution, syrup, and dripping pill dosage forms.

For injection, it is possible to use its solid or liquid preparation dose, e.g., powdered injection dose, solution injection and others.

In order to more fully understand the nature of this invention, the present invention's preparation methods (below) to obtain the plant ethanol extract described above, the pharmacological action of the extracted effective part (abbreviated below as PUW), and its results in a pharmaceutically acceptable carrier or excipient compound medication are used to illustrate its applications in the medical field.

1. PAI-1 inhibiting activity filter test

r-PAI-1 is a product from Molecular Innovations Inc. in the United States, urokinase is a Sigma product, and Chromozym U is a product from Boehringer Mannheim of Germany. The test sample was dissolved at fixed concentration in DMSO, first incubated 10 min with r-PAI-1, then urokinase and substrate Chromozym U added, the reaction started; after 45 min, 32% acetic acid was used to interrupt the reaction, and the substrate decomposition product's absorption value was determined at a wavelength of 405 nm. Corilagin IC_{50} measured using the test was 10 μg/mL.

2. Murine acute toxicity test

A: Oral medication administration: ICR mice, half male, half female, weight 18-22g , fasted for 12 h, 0.4 mL/10 g PUW was administered by gastric perfusion, because the half-lethal dose could not be found, the test was performed using maximum tolerated dose, the mice were continuously observed for 14 days, and not one mouse died, their maximum tolerated dose (MTD) was 5.6 g/kg.

A. Intravenous injection: ICR mice, half male, half female, weight 18-22 g, fasted for 12 h, 0.2 mL/10 g PUW was injected intravenously through the tail over 20 sec. Mice were continuously observed for 14 days, half-lethal dose was 259.6 mg/kg (95% confidence interval: 222.4-303.1 mg/kg).

3. Rat tail tip bleed test

After 10 mg/kg PUW were injected once intravenously into male SD rat thigh, the bleed time at the tip of the animal's tail for the physiological saline solution group extended slightly from 14.6 ± 0.6 min to 16.9 ± 1.3 min, and after intravenous injection of 100 U/kg heparin, the bleed time clearly extended to /4 37.1 ± 1.3 min. It demonstrated that the stronger the PUW anti-embolic action, the less it affected bleeding.

4. Impact of PUW on arachidonic acid-induced thrombopoiesis in mice

A. Oral medication administration: At the tail vein, male mice were injected with an aliquot of arachidonic acid (AA). The death status of the mice was observed for 30 min to assess the influence of the medication on the death rate in conscious animals *in vivo* with thrombopoiesis activity. 75 mg/kg AA was injected into the tail vein. For noteworthy cases, χ^2 test processing was performed. The results indicated that the activity of 10 mg/kg PUW was identical to that of 20 mg/kg aspirin, and the death rate was reduced from 80% in the control group to 53.3%.

A. Intravenous injection: After intravenous injection with 2.5 mg/kg PUW, animal death count ($P >$ 0.05) was clearly reduced, after intravenous injection of 5 mg/kg PUW, its activity was equal to that of 10 mg/kg, and the death rate was reduced from 80% in the control group to 40%.

5. Impact of PUW on rat carotid thrombopoiesis induced by electrical stimulation

Male SD rats, weighing 250-300 g. The left carotid artery in all animals was exposed and silver electrodes used to perform stimulation (distance of 1 cm between the positive and negative electrodes) at

1.5 mA for 7 min and carotid artery blood flow was detected using a blood flow meter. Time from start of stimulation until blood flow reached zero was the thrombopoiesis time (occlusion time, OT).

A. Orally administered medication: OT for the physiological saline solution group was 18.1 ± 1.6 min, for the 20 mg/kg aspirin group, it was 34.2 ± 2.3 min, and for the PUW 10 and 20 mg/kg groups, OT was 27.0 ± 2.6 min and 35.0 ± 3.6 min.

A. Intravenous injection: OT for the physiological saline solution group was 17.7 ± 0.8 min, for the 20 mg/kg aspirin group, it was 27.8 ± 1.6 min, for the PUW 5, 10, and 20 mg/kg groups, the OTs were, respectively, 27.2 ± 1.7, 31.2 ± 2.1, and 36.7 ± 1.9 min. The results demonstrate that PUW at 5 mg/kg had a clear activity against electrically-stimulated rat carotid artery thrombopoiesis; its strength was identical to that of 20 mg/kg aspirin.

6. Venous thrombopoiesis activity

Male SD rats weighing 200-250 g. 40 mg/kg sodium pentobarbital anesthesia was administered and the abdominal cavity opened along the midline of the abdomen, the inferior vena cava freed and tied at the level of the left renal vein, then the abdominal cavity was closed. After 1 h, the medication was administered through gastric perfusion or through the femoral vein; again 1 h passed, the abdominal cavity was opened, clots were collected within the inferior vena cava, wet weight was obtained, then baked for 20 h in a 60°C roasting box and its dry weight obtained.

A. Orally administered medication: physiological saline solution group dry and wet thrombus weights were, respectively, 4.1 ± 0.8 and 9.9 ± 2.0 mg. For the 20 mg/kg aspirin group they were, respectively, 1.0 ± 0.9 and 2.2 ± 2.3 mg. For the 20 mg/kg PUW group, dry and wet thrombus weights were 1.3 ± 0.9 and 2.9 ± 2.0 mg. /5

A. Intravenous injection: Dry and wet thrombus weights for the physiological saline solution group were, respectively, 4.1 ± 0.5 and 9.9 ± 1.8 mg. 10 mg/kg aspirin group dry and wet thrombus weights were, respectively, 2.9 ± 0.5 and 6.1 ± 1.3 mg. For the 5, 10 and 20 mg/kg PUW groups, dry thrombus weights were, respectively, 2.8 ± 0.6, 2.2 ± 0.7 and 1.7 ± 0.6 mg, at the above-described PUW doses, thrombus wet weights were, respectively, 6.0 ± 1.1, 5.2 ± 0.9, and 3.7 ± 1.0 mg.

7. PUW's thrombolytic action: Male SD rats, weighing 200-300 g, the modified Peter method employed, the left carotid artery stimulated using 2 mA for 5 min, and a blood flow meter was used continuously to detect carotid artery blood flow. After the stimulation concluded, 50% pre-stimulation blood flow rate measured was the thrombopoiesis time. 20 min after thrombopoiesis, PUW, physiological saline solution and urokinase were all intravenously injected. 1 h after the medication was administered, vascular recanalization was observed. If recanalization occurred, then vascular openness was continuously observed for 1 h. Using pre-stimulation blood flow rates ≥ 50% or ≤ 25% to define recanalization or subsequent reembolization, the degrees of vascular openness, respectively, were no recanalization (P0), recanalization and reembolization crisscrossing (CR), and persistent post-recanalization openness (PP). The results demonstrated that PUW had a dose-related thrombolytic effect: at 5 mg/kg, the recanalization rate was equal to that of 2000 U/kg urokinase; at 10 mg/kg, the recanalization rate was 50%, it was lower than the 60% reembolization rate of 2000 U/kg urokinase; not one animal in the physiological saline solution group showed recanalization; the state of vascular openness after recanalization was: all rats in the physiological saline solution group had persistent embolisms; for those receiving 2000 U/kg urokinase, the openness was similar to that of those receiving 5 mg/kg PUW; in the 10 mg/kg PUW group, the persistent recanalization rate was higher than for the 2000 U/kg urokinase (for vascular openness, see Table 1). The above results revealed that after one

intravenous injection, PUW had stronger thrombolytic action and prevented reembolization after thrombolysis.

TABLE 1. Arterial Openness after One Intravenous Injection of PUW Thrombolytic.

①药物	②剂量（mg/kg）	③血管开放状态分值		
		④持续栓塞	⑤栓塞与再通交错	⑥持续再通
⑦生理盐水	2ml/kg	8	0	0
PUW	2.5	4	3	1
	5.0	3	3	2
	10.0	2	3	3
⑧尿激酶	2000U/kg	3	4	1

⑨ *n*=8 雄性大鼠

Key: 1 Medication

2 Dose

3 Sub-values for vascular openness

4 Continued embolism

5 Embolism and recanalization crisscrossing

6 Sustained recanalization

7 Physiological saline solution

8 Urokinase

9 *n* = 8 male rats

TABLE 2. PUW Thrombolytic Action against Arterial Thrombosis. /6

① 药物	② 剂量 (mg/kg)	③ 血栓形成时间 (min)	④ 血流量零点时间(min)	⑤ 再通数/总数	⑥ 再栓数/再通数
⑦ 生理盐水	2ml/kg	7.5±3.1	18.8±3.6	0/8	0/0
PUW	2.5	7.9±2.7	20.8±3.9	3/8*	2/3*
	5.0	8.1±3.4	18.7±4.3	5/8*	3/5*
	10.0	7.7±3.5	19.5±4.0	6/8*	3/6*
⑧ 尿激酶	2000U/kg	7.4±3.3	19.2±4.4	5/8*	3/5*

⑨ n=8 雄性大鼠，x±s，*P<0.05 (χ^2 test)与生理盐水比较。

Key: 1 Medication

2 Dose

3 Thrombopoiesis

4 Blood flow rate zero point time

5 Recanalization count/total

6 Reembolization count/recanalization count

7 Physiological saline

8 Urokinase

9 $n = 8$ male rats, $x \pm s$, $^*P < 0.05$ (χ^2 test) compared to physiological saline solution.

The pharmacokinetic results above demonstrate that oral and intravenous injection of PUW had a strong thrombolytic and anti-thrombopoietic action in many thrombosis models.

8. Mechanism of action

PUW *in vitro* showed increased tissue-type plasminogen activator (tPA) activity and reduced type-1 plasminogen activator inhibitor (PAI-1) activity. *In vivo* testing: after rats were anesthetized, they

received intravenous injection of 1.0 mg/kg/min PUW for 20 min, which clearly reduced PAI-1 activity in plasma and platelets and elevated tPA activity, indicating that PUW initiates anti-thrombosis action by suppressing PAI-1 activity and elevating tPA activity.

As described above, PUW serves as a novel thrombolytic medication and has the following advantages:

1) *Phyllanthus urinaria*, and from the same plant genus, *Phyllanthus cochinchinensis* or *Phyllanthus emblica* or *Phyllanthus simplex* or *Phyllanthus matsumurae* are plentiful and cheap. Their extraction and processing technologies are simple and they can be manufactured in batch quantities.

2) Both oral and intravenous injection are possible, the thrombolytic pharmacokinetics are confirmed, and the activity is strong.

3) It overcomes the hemorrhagic side effects easily produced with common thrombolytic medications now in clinical use.

4) There is no toxicity after oral and intravenous injection.

5) Its thrombolytic mechanism of action *in vivo* and *in vitro* is to directly inhibit PAI-1 activity and elevate tPA activity, and to date it is the only plant-sourced natural small-molecule PAI-1 inhibitor.

6) It supplies a new thrombolytic medication that prevents, treats, and achieves recovery from /7
myocardial infarction, hypertension, angina pectoris, stroke, cerebral embolism, pulmonary embolism, limb arteriovenous embolism, retinal arteriovenous embolism, and other diseases.

The application examples gathered below serve to more substantially illustrate the nature of the present invention; however they do not serve to limit the present invention.

Application Example 1

Fresh *Phyllanthus urinaria* is harvested from the ground, partly air dried, and pulverized to obtain 15.42 kg of dry powder. Then it is extracted 4 times using 93% or higher industrial alcohol at room temperature, the extraction solution vacuum condensed at 40°C and dried to obtain 1365 g extract. The extract is dissolved and suspended in the n-butyl alcohol layer of n-butyl alcohol-acetic acid-alcohol (4:1:5). Aqueous layer extraction is performed and the water layers merged, vacuum condensed at 40°C to a small volume, then spray drying performed to obtain 401.7 g dry powder (PUW). Using high-pressure liquid chromatography (HPLC), the dry powder (PUW) is tested, there are two main ingredients, of which the corilagin level is higher than 60% (pure corilagin is used as the standard control, levels measured by use of routine HPLC methods). The excipient is added at a dry powder to excipient weight ratio of 9:1, and prepared into a capsule.

Application Example 2

Phyllanthus urinaria dry powder (PUW) is first prepared according to the methodology in Application Example 1, the excipient is added at a dry powder to excipient weight ratio of 5:1 and prepared into a powder dose.

Application Example 3

Phyllanthus urinaria dry powder (PUW) is first prepared according to the methodology in Application Example 1, the excipient is added at a dry powder to excipient weight ratio of 5:1 and prepared into a sugar-coated dose.

Application Example 4

Phyllanthus urinaria dry powder (PUW) is first prepared according to the methodology in Application Example 1, the excipient is added at a dry powder to excipient weight ratio of 2:1, and the grains pressed into tablets.

Application Example 5

Fresh *Phyllanthus urinaria* is harvested from the ground, partly air dried, and pulverized to obtain 15.42 kg of dry powder. Then it is extracted 3 times using 93% or higher industrial alcohol at room temperature, the extraction solution is vacuum condensed at 35°C until dry to obtain 1365 g extract. The extract is dissolved and suspended in the n-butyl alcohol layer of n-butyl alcohol-acetic acid-alcohol (4:1:5). Aqueous layer extraction is performed and the water layers merged, it is vacuum condensed at 40°C to a small volume, then spray drying performed to obtain 401.7 g dry powder (PUW). Using high-pressure liquid chromatography (HPLC), the dry powder (PUW) is tested, there are two main ingredients, of which the corilagin level is higher than 60% (pure corilagin is used as the standard control, levels are measured by use of routine HPLC methods), the dry powder is dissolved in sterile water for injection, stirred to dissolve, a sterile filter funnel used to perform filtration, sterile precision filtration performed again, and the product packaged into 2-mL ampules. After cooling and drying at low temperature, sterile sealing by fusion is performed to obtain the dry injection dose. /8

Application Example 6

Phyllanthus urinaria (PUW) is first prepared into a dry powder according to the method in Application Example 1 or 5, then prepared into an oral solution by routine oral solution preparation methods.

Application Example 7

Phyllanthus urinaria (PUW) is first prepared into a dry powder according to the method in Application Example 1 or 5, then the dry powder is dissolved in aqueous solution and prepared into a syrup by routine syrup preparation methods.

Application Example 8

Phyllanthus urinaria (PUW) is first prepared into a dry powder according to the method in Application Example 1 or 5, then it is prepared into a dripping pill dosage form using routine dripping pill preparation methods.

Application Example 9

Phyllanthus urinaria (PUW) is first prepared into a dry powder according to the method in Application Example 1 or 5, then an injection-use solution is prepared using routine preparation methods, then precision filtered, filled, sealed and sterilized to prepare into an injection-use solution.

Application Example 10

Fresh *Phyllanthus cochinchinensis urinaria* is harvested from the ground, partly air dried, and pulverized to obtain 1 kg dry powder. Then it is extracted 4 times using 93% or higher industrial alcohol at room temperature, the extraction solution is vacuum condensed at 40°C and dried to obtain 90 g extract. The extract is dissolved and suspended in the n-butyl alcohol layer of n-butyl alcohol-acetic acid-water (4:1:5). Aqueous layer extraction is performed and the water layers merged, the extraction solution is vacuum condensed at 40°C to a small volume, and then spray drying is performed to obtain

25 g dry powder. Using high-pressure liquid chromatography (HPLC), the dry powder (PUW) is tested, and there are two main ingredients, of which the corilagin level is higher than 60% (pure corilagin is used as the standard control, levels are measured by routine HPLC methods). The excipient is added at a dry powder to excipient weight ratio of 9:1 and prepared into a capsule.

Application Example 11

From the same plant genus, fresh *Phyllanthus emblica* leaves are collected, air dried, and pulverized to obtain 5 kg dry powder. Then it is extracted 4 times using 93% or higher industrial alcohol at room temperature, the extraction solution is vacuum condensed at 40°C and dried to obtain 450 g extract. The extract is dissolved and suspended in the n-butyl alcohol layer of n-butyl alcohol-acetic acid-water (4:1:5). Aqueous layer extraction is performed and the water layers merged, the extraction solution /9
vacuum condensed at 40°C to a small volume, then spray drying performed to obtain 150 g dry powder. Using high-pressure liquid chromatography (HPLC), the dry powder (PUW) is tested, there are two main ingredients, of which the corilagin level is higher than 60% (pure corilagin is used as the standard control, levels are measured by routine HPLC methods). The excipient is added at a dry powder to excipient weight ratio of 9:1 and prepared into a capsule.

Application Example 12

Fresh *Phyllanthus simplex* is harvested from the ground, partly air dried, and pulverized to obtain 10 kg dry powder. Then it is extracted 4 times using 93% or higher industrial alcohol at room temperature, and the extraction solution vacuum condensed at 40°C and dried to obtain 985 g extract. The extract is dissolved and suspended in the n-butyl alcohol layer of n-butyl alcohol-acetic acid-water (4:1:5). Aqueous layer extraction is performed and the water layers merged, the extraction solution

vacuum condensed at 40°C to a small volume, then spray drying performed to obtain 280 g dry powder. Using high-pressure liquid chromatography (HPLC), the dry powder is tested, there are two main ingredients, of which the corilagin level is higher than 60% (pure corilagin is used as the standard control, levels measured by routine HPLC methods). The excipient is added at a dry powder to excipient weight ratio of 9:1 and prepared into a capsule.

Application Example 13

1 kg of whole-herb plant from the same genus *Phyllanthus matsumurae* is collected, air dried, pulverized and water added to decoct 2 times. The water solution is processed through a large-pore adsorption resin to obtain total glycoside, the total glycoside is passed through a silica gel short column to precipitate coarse fractionation and again undergoes purification to obtain 5.07 g of beta-1-O-galloyl-3,6-(R)-hexahydroxydiphenoyl-D-glucose (corilagin) compound. Excipient is added at a corilagin to excipient weight ratio of 9:1 and prepared into a capsule.